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SUCROSE ABSORPTION BY THE RAT SMALL INTESTINE *IN VIVO* AND *IN VITRO*

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SUMMARY

1. The absorption of glucose and fructose derived from sucrose has been studied using *in vitro* and *in vivo* loops of the rat jejunum.

2. At low sucrose concentrations (1 and 10 mM) glucose appeared in the serosal compartment of the *in vitro* preparation at a faster rate than fructose, but at high sucrose concentrations (50 and 100 mM) the rates of serosal transfer of the two sugars were similar. Glucose and fructose appeared in the mucosal compartment, with the rate of fructose appearance exceeding that of glucose, at all the sucrose concentrations studied.

3. Phlorizin (5×10^{-5} M) added to the mucosal medium of the *in vitro* preparation abolished the serosal transfer of glucose derived from 50 mM sucrose, and reduced that of fructose by 75 %.

4. In the absence of sodium ions, the *in vitro* preparation failed to transfer glucose and fructose derived from 50 mM sucrose, into the serosal compartment.

5. Glucose was actively accumulated in the whole gut wall of the *in vivo* preparation to concentrations higher than those in the plasma at 50 and 100 mM, but not at 10 mM sucrose concentrations. Fructose was also actively accumulated to about half the extent of glucose, but reached tissue concentrations greater than those in the plasma, at each sucrose concentration.

6. The whole wall concentrations of glucose and fructose derived from sucrose added to the lumen continued to rise when the blood supply to the *in vivo* preparation was terminated.

7. No increase in the *in vivo* whole wall concentrations of glucose and fructose were detected when sucrose was added to the lumen together with concentrations of glucose sufficient to saturate the monosaccharide transport systems.

8. The results favour the view that disaccharide hydrolysis and resulting hexose transfer are sequential, separate events.

INTRODUCTION

While the absorption of monosaccharides by the small intestine has been studied extensively, the absorption of the principal disaccharides, maltose, lactose and sucrose, has attracted less attention, although these are major products of carbohydrate digestion. Studies on the transport of sucrose across the small intestine have mainly been concerned with the glucose rather than the fructose moiety, despite evidence that the fructose stemming from sucrose elevates plasma triglyceride levels (Naismith, 1971; Macdonald, 1971), and may predispose individuals to ischaemic heart disease and obesity (Yudkin, 1968). We have therefore examined in detail the absorption of glucose and fructose arising from sucrose, using *in vitro* and *in vivo* preparations of the rat jejunum.

METHODS

Male Wistar rats of 200–250 g body weight that had been allowed free access to food were used in all the experiments. The apparatus used for both the *in vivo* and *in vitro* experiments was similar to that described by Fisher & Parsons (1949). This is a preparation in which the perfusion medium is driven round the lumen of the gut by a gas-lift in which bubbles of 95 % O_2/5 % CO_2 (v/v) perform the dual function of recirculation and oxygenation. The rats were anaesthetized with ether, the body cavity opened, approximately 30 cm of jejunum exposed and the gut contents gently flushed out with ice-cold 0·9 % NaCl. The segment was cannulated and connected to the mucosal circuit of the apparatus. The perfusion medium was a modified Krebs bicarbonate Ringer (Bronk & Parsons, 1965) containing NaCl 118 mM, $NaHCO_3$ 25 mM, KCl 4·74 mM, $MgSO_4$ 1·19 mM, KH_2PO_4 1·17 mM, $CaCl_2$ 1·70 mM, equilibrated with 95 % O_2/5 % CO_2 (v/v). All incubations were carried at 37° C.

In vitro experiments

In the *in vitro* experiments, the mesenteries were cut, the segment was rinsed of surface blood, gently blotted on tissue paper, and placed in a serosal chamber containing perfusion medium oxygenated and circulated by a stream of 95 % O_2/5 % CO_2 (v/v). The initial volume of the mucosal and serosal solutions was 50 ml. Various concentrations of sucrose were added to the mucosal medium after a 10 min preincubation period, and the experiments continued for a further 50 min unless otherwise stated. During this time, serial samples of the mucosal and serosal fluids were taken for sugar analysis. At the end of the experiments, the final mucosal and serosal volumes were measured so that corrections for fluid movements could be made when calculating the absolute amounts of sugar transfer.

In vivo experiments

The method has been described by Leese (1974). Approximately 30 cm of the jejunum of anaesthetized rats was cannulated and perfused through the lumen with the mesentery intact, using the mucosal circuit of the Fisher & Parsons apparatus. The jejunum was replaced inside the body cavity during perfusion and the rat maintained under ether. After 5 min, various concentrations of sucrose were

added to the mucosal fluid, and the perfusions continued for a further 10 or 25 min. At the end of the experiments, a sample of mucosal medium was taken, the segment re-exposed and about 5 cm clamped between aluminium tongs pre-cooled in liquid nitrogen (Wollenberger, Ristau & Schoffa, 1960). The frozen segment was held in liquid nitrogen while approximately 1 ml. blood was withdrawn from the heart into tubes containing EDTA. The blood was centrifuged to separate the plasma and the concentrations of glucose and fructose in this and the mucosal and tissue samples were determined.

Analytical procedures

Glucose and fructose were determined on the same samples by a modification of the automated procedure of Leese & Bronk (1972). In this method, glucose is converted to 6-phosphogluconic acid in coupled reactions catalysed by hexokinase and glucose-6-phosphate dehydrogenase, so that the amount of NADPH formed from NADP, which is estimated fluorimetrically, is proportional to the amount of glucose in the sample. Fructose was estimated by re-running the samples in the presence of a third enzyme, phosphoglucose isomerase, which resulted in the production of an extra increment of NADPH, proportional to the fructose in the sample. In preliminary experiments, it was shown that the amounts of NADPH produced when the two sugars were estimated individually were identical with those produced when they were run as a mixture. The sucrose used in the experiments was found not to be contaminated with glucose or fructose, nor to interfere in the analysis of its constituent monosaccharides.

Expression of results

Results for the *in vitro* experiments were expressed as μmole sugar transferred/cm intestine as measured at the end of the experiment. The results for the *in vivo* glucose and fructose concentrations were expressed as μmole/ml. (i.e. mM) as described by Leese (1974).

RESULTS

In vitro experiments

A number of experiments were carried out with 1, 10, 50 and 100 mM sucrose added to the mucosal fluid. In each case, a corresponding amount of NaCl was omitted from the mucosal fluid to preserve the original tonicity of this medium. The appearance of glucose and fructose was monitored in the mucosal and serosal fluids, and the results are shown in Fig. 1(*A–D*). Glucose and fructose appeared in the mucosal medium at all concentrations studied. At 1 mM sucrose, the rates were roughly comparable, but at 10, 50 and 100 mM sucrose, fructose appeared at much greater rates than glucose. The amounts of glucose and fructose appearing in the serosal medium were very small at 1 mM sucrose, but increased with increasing sucrose concentration. The glucose in the serosal fluid at 10 min (i.e. before sucrose addition) was probably derived from blood washed out of the cut mesenteric vessels. The rates of serosal appearance of the two sugars at 50 and 100 mM sucrose were comparable, but glucose appeared at about four times the rate of fructose with 10 mM sucrose.

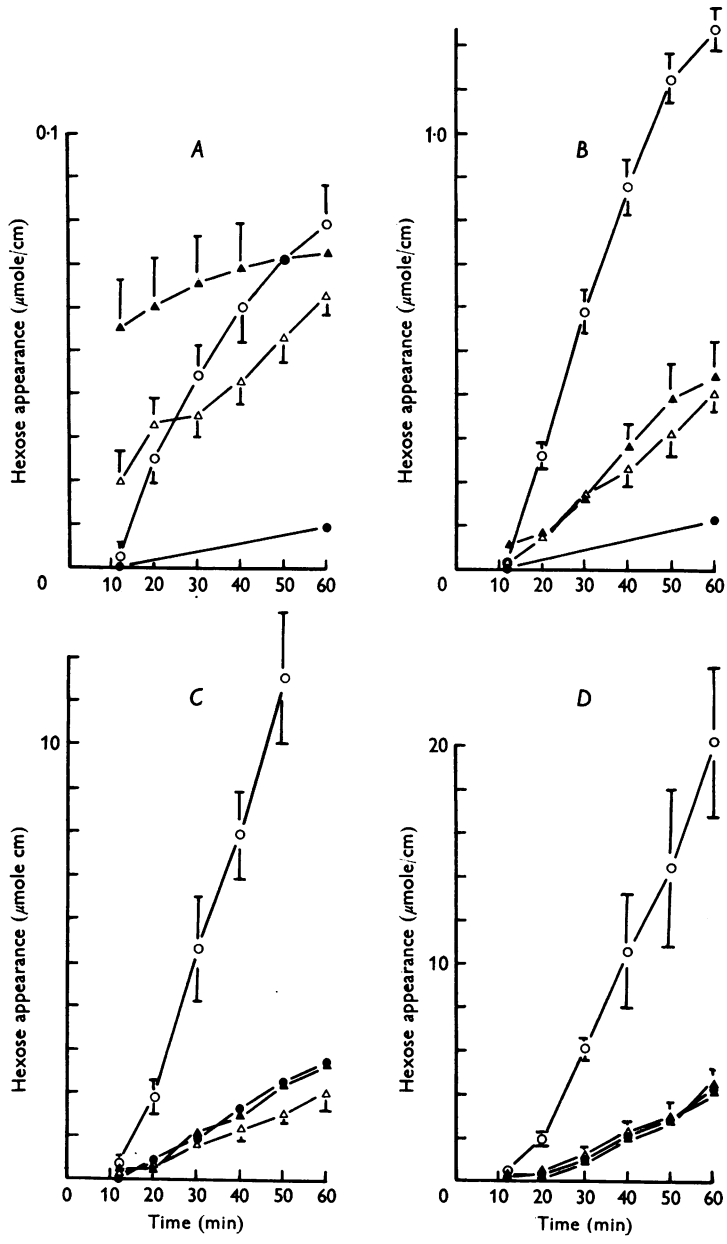

Fig. 1. Effect of increasing concentrations of sucrose on the rates of appearance of glucose and fructose in the mucosal and serosal fluids of an *in vitro* preparation of the rat jejunum. Sucrose added to the mucosal fluid, after 10 min preincubation, to a concentration of 1 mM (*A*), 10 mM (*B*), 50 mM (*C*) and 100 mM (*D*). △, rate of appearance of glucose in mucosal fluid. ○, rate of appearance of fructose in mucosal fluid. ▲, rate of appearance of glucose in serosal fluid. ●, rate of appearance of fructose in serosal fluid. Values are means ± S.E. of mean of at least four observations. Note that the scale of the ordinate increases from Fig. 1*A* to *D*.

Experiments were carried out to investigate the effect of phlorizin, which is commonly used to inhibit monosaccharide active transport. Segments of jejunum were preincubated for 10 min with phlorizin (5×10^{-5} M) added to the mucosal medium, before the addition of sucrose to a concentration of 50 mM. Fig. 2 shows that phlorizin virtually abolished the serosal transfer of glucose stemming from sucrose and reduced that of

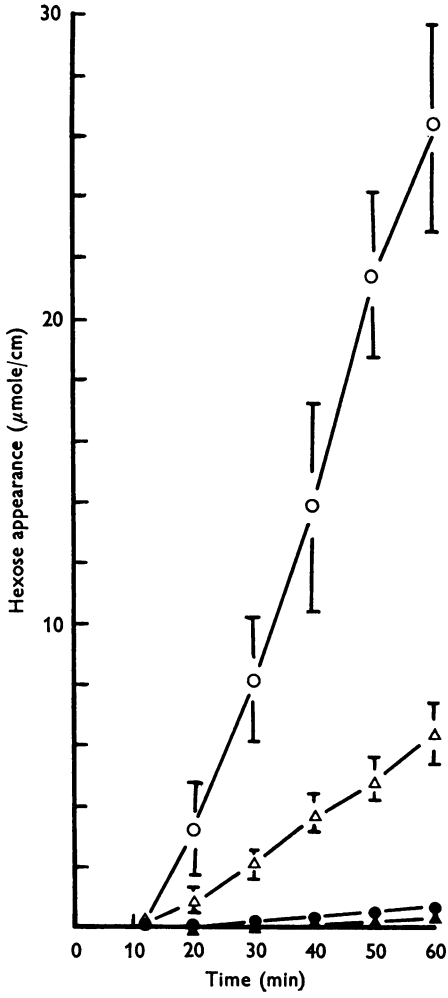

Fig. 2. Effect of phlorizin (5×10^{-5} M added to the mucosal fluid) on the rates of appearance of glucose and fructose in the mucosal and serosal fluids of an *in vitro* preparation of the rat jejunum. Sucrose to a concentration of 50 mM was added to the mucosal fluid after 10 min preincubation. Symbols as in Fig. 1. Values are means ± S.E. of mean of four observations.

fructose by about 75%. The mucosal appearances of glucose and fructose were correspondingly elevated about threefold (for control, see Fig. 1*C*).

The effect of omitting sodium ions, and to a large extent chloride ions, from both the mucosal and serosal fluids was examined. For these experiments, the segment of intestine was rinsed with isotonic KCl rather than isotonic saline. The NaCl and $NaHCO_3$ in the perfusion medium were replaced with isosmolar quantities of mannitol and $KHCO_3$ respectively.

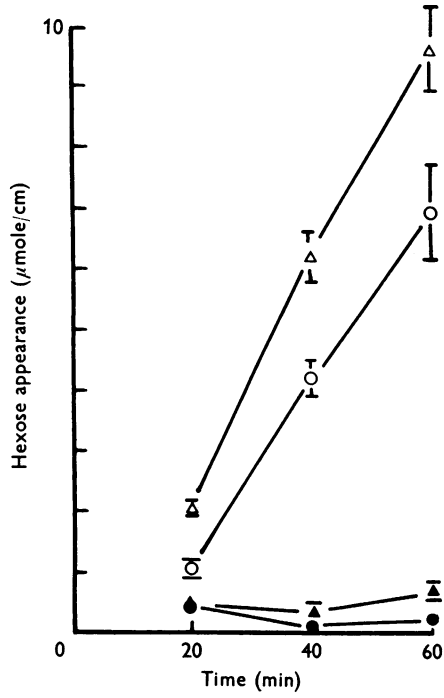

Fig. 3. Effect of omitting Na ions, and to a large extent Cl ions, from the incubation media, on the rates of appearance of glucose and fructose in the mucosal and serosal fluids of an *in vitro* preparation of the rat jejunum. Sucrose to a concentration of 50 mM was added to the mucosal fluid after 10 min preincubation. Symbols as in Fig. 1. Values are means ± S.E. of mean of four observations.

Sucrose was again added at 10 min to a concentration of 50 mM and the data is presented in Fig. 3. Na replacement abolished glucose and fructose transfer into the serosal fluid. The rate of appearance of glucose in the mucosal medium was increased over fourfold, while that of fructose was reduced by about one half. As a result the rate of mucosal glucose appearance now exceeded that of fructose. Semenza (1968) has shown that sucrase isolated from a number of species is activated by sodium ions, and

it was therefore of interest to observe that sucrose was hydrolysed to glucose and fructose even in the absence of Na ions in the perfusing media.

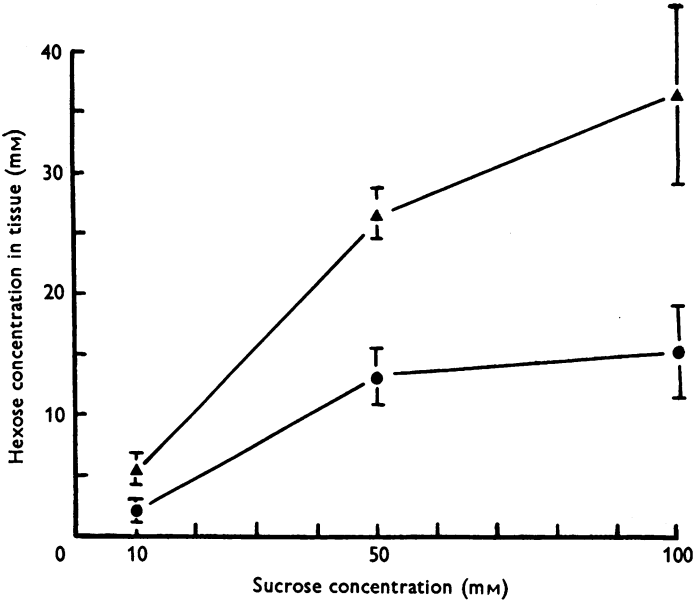

Fig. 4. Effect of increasing sucrose concentration on the glucose and fructose concentrations in whole segments of rat jejunum, incubated *in vivo*. The incubation time was 15 min with sucrose being added to the mucosal fluid at 5 min. ▲, glucose concentration in tissue water. ●, fructose concentration in tissue water. Values are means ± S.E. of mean of four observations.

In vivo experiments

Glucose and other monosaccharides are actively accumulated by *in vitro* preparations of the small intestine, and evidence in favour of accumulation *in vivo* has also been obtained (Leese, 1974). It was therefore of interest to see whether the glucose and fructose stemming from sucrose were accumulated in the gut wall during absorption *in vivo*. Experiments were carried out with 10, 50 and 100 mM sucrose perfused through the lumen for 10 min with the blood supply to the gut intact. The final concentrations of glucose and fructose in the tissue water are given in Fig. 4. Similar results were obtained with 30 min perfusions, suggesting that a steady state had been reached after 10 min. Glucose was actively accumulated by the tissue, and reached concentrations higher than those in the plasma at 50 and 100 mM sucrose, but not at 10 mM sucrose. Fructose was also actively accumulated, to a lesser extent than glucose, but reached tissue concentrations greater than those in the plasma at each

sucrose concentration. In all these experiments the concentration of glucose and fructose in the mucosal medium never exceeded 1·5 mM. After *in vivo* perfusion of a mixture of glucose and fructose, each at a concentration of 50 mM the tissue fructose concentration was 9·9 ± 1·7 (4) mM, which is roughly comparable with that obtained with 50 mM sucrose (13·0 ± 1·9 (4)), but the glucose concentration, at 44·9 ± 2·6 (4) mM was about 70% higher than that obtained with sucrose (26·4 ± 1·5 (4) mM).

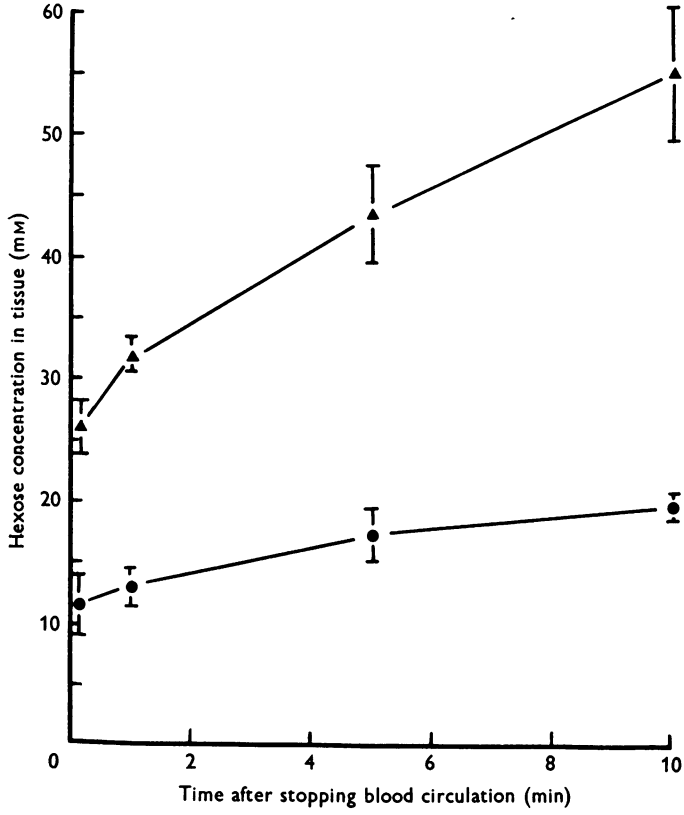

Fig. 5. Effect of lack of blood circulation on the glucose and fructose concentrations in whole segments of rat jejunum incubated *in vivo*. Segments were perfused through the lumen with 50 mM sucrose and intact mesentery for 30 min. The heart was then cut, and perfusion continued for the times shown. Symbols as in Fig. 4. Values are means ± S.E. of mean of four observations.

With the advent of preparations for the vascular perfusion of isolated amphibian and mammalian small intestine (e.g. Parsons & Prichard, 1968; Hanson & Parsons, 1976), the importance of the effective clearance

of absorbed nutrients from the serosal surfaces of the epithelial cells has become apparent. This was highlighted by a series of experiments in which an *in vivo* perfusion was carried out with 50 mM sucrose for 30 min, and the blood circulation then stopped by cutting the heart. The luminal circulation was continued, and segments of gut freeze-clamped at 1, 5 and 10 min after cessation of the circulation. The results of these experiments are given in Fig. 5. Both glucose and fructose continued to be accumulated by the tissue above their *in vivo* concentrations, the glucose markedly so.

Malathi, Ramaswamy, Caspary & Crane (1973) and Ramaswamy, Malathi, Caspary & Crane (1974) showed that under *in vitro* conditions, in which the glucose transport system was maximally active, the addition of sucrose or other disaccharides led to an increased uptake of glucose, i.e. that there was a glucose uptake from the disaccharides which did not utilize the monosaccharide carrier. Experiments were performed to determine whether or not this occurred in the rat jejunum *in vivo*. Segments were perfused through the lumen with intact mesentery for 15 min with 50 and 100 mM glucose. The whole wall concentrations of glucose as measured in freeze-clamped segments were $40{\cdot}4 \pm 2{\cdot}6$ (3) mM and $37{\cdot}6 \pm 4{\cdot}8$ (3) mM respectively, suggesting that the glucose transport system was saturated at these glucose concentrations. Segments of jejunum were then perfused for 15 min with a mixture of 100 mM glucose and 50 mM sucrose. The concentration of glucose in the tissue under these conditions was $39{\cdot}5 \pm 0{\cdot}77$ (3) mM which was therefore similar to that obtained with glucose alone, suggesting that a sucrase-mediated glucose transport system was not operating.

DISCUSSION

The present findings have confirmed that the glucose and fructose derived from sucrose are readily absorbed by an *in vitro* preparation of the rat jejunum, and are accumulated in the gut wall of an *in vivo* preparation. When 1 mM sucrose was perfused *in vitro*, only small amounts of glucose and fructose were detected in the mucosal fluid whereas with 10, 50 and 100 mM sucrose, the amount of free monosaccharides appearing in the mucosal fluid rose rapidly, with considerably more fructose being released than glucose (Fig. 1*A–D*). Since equal amounts of glucose and fructose are liberated by the enzyme sucrase, this indicates that the glucose stemming from sucrose was absorbed at a faster rate than fructose. It was therefore reasonable to expect more glucose to appear in the serosal fluid than fructose. This was the case with 1 and 10 mM sucrose, but with 50 and 100 mM sucrose, the rates of appearance of the two sugars in the serosal fluid were similar. This could mean that the mechanism for transporting glucose stemming from sucrose was more active than that

for transporting fructose at low sucrose concentrations, but that at high sucrose concentrations, glucose and fructose were transported with equal affinity. Alternatively, or in addition, glucose could be taken up in preference to fructose at the brush border pole of the epithelial cells at all the sucrose concentrations studied, but be metabolized or retained in the tissue to a greater extent than fructose at high sucrose concentrations so that its rate of transport into the serosal fluid was reduced to that of fructose. This could account for the discrepancy at high sucrose concentrations between the amounts of glucose and fructose liberated into the mucosal medium, and the amounts appearing in the serosal medium. It is also in agreement with the finding (Fig. 5) that glucose was accumulated in the gut wall to a concentration over three times that of fructose. If account is taken of differing sucrose concentrations, our results for sugar transport are substantially in agreement with those of Chain, Mansford & Pocchiari (1960), who used a similar *in vitro* preparation to study the absorption of sucrose at a concentration of 14·6 mM.

There are two main ideas as to the relationship between disaccharide hydrolysis and the transfer of the liberated monosaccharides. Until recently, the evidence favoured the view that disaccharide hydrolysis and hexose transport were sequential, separate events, but that hexose released from disaccharides had a kinetic advantage for transport over hexose free in the lumen (Parsons & Prichard, 1971). In this scheme, hexose liberated from disaccharides was transported by the conventional sodium-dependent monosaccharide transport system(s). An alternative scheme has recently been proposed (Malathi *et al.* 1973; Ramaswamy *et al.* 1974; Storelli, Vögeli & Semenza, 1972) in which the disaccharidase is vectorial, i.e. in the case of sucrase, is able both to hydrolyse sucrose and transfer the resulting glucose and fructose in an intracellular direction. The present results are better interpreted in terms of the first mechanism. In the absence of Na, or the presence of phlorizin, the serosal transfer of glucose and fructose stemming from sucrose was abolished. The disaccharidase-related transport system is substantially independent of sodium and less sensitive than the monosaccharide system to phlorizin (Malathi *et al.* 1973; Ramaswamy *et al.* 1974) and some capacity to transfer glucose stemming from sucrose would have been expected if the disaccharidase-related system had been operative under these conditions. It is interesting to note that some capacity to transfer fructose from sucrose was preserved in the presence of phlorizin. This may partly be accounted for by the presence of a distinct D-fructose transport system, insensitive to this inhibitor (Sigrist-Nelson & Hopfer, 1974). We failed to detect an increase in whole gut glucose and fructose concentrations *in vivo* in experiments where sucrose was added together with concentrations of glucose sufficient

to saturate the monosaccharide transport system which is further evidence against a specific sucrase-mediated transport system. However, in any consideration of the alternative mechanisms, it should be borne in mind that the disaccharidase-related transport systems are probably of minor physiological significance, accounting for the transport of not more than 5–10% of the monosaccharides liberated from disaccharides (Semenza, 1975). This might make the detection of these systems difficult under the present experimental conditions.

The tissue concentrations of glucose and fructose after perfusion of sucrose *in vivo* were very much higher than those measured in the mucosal fluid (Fig. 4), indicating that both sugars were subject to an active transport process. This is in line with the early work of Miller & Crane (1961) on hamster tissue, as confirmed by Ramaswamy *et al.* (1974), and of Gracey, Burke & Oshin (1972), who reported that fructose presented as the monosaccharide is accumulated over twofold by the rat small intestine *in vitro*.

Finally, we would like to draw attention to an important difference between an *in vivo* and an *in vitro* preparation of the rat small intestine. Under *in vivo* conditions, nutrients taken up at the brush border pole of the epithelial cells are rapidly cleared from the mucosa via the mesenteric circulation and their accumulation in the gut wall is limited. In all *in vitro* preparations with the exception of vascular perfusion, the mesentery is severed from the rest of the circulation and the physiological clearance of nutrients from the serosal surfaces of the mucosa is restricted.

As the data in Fig. 5 illustrate, the combination of a continuing uptake of sugar from the mucosal medium with a diminished clearance leads to the retention of nutrients in the gut wall at concentrations much higher than those ever likely to occur *in vivo*.

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